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Relationship between Agronomic Parameters, Phenolic Composition of Grape Skin, and Texture Properties of *Vitis vinifera* L. cv. Tempranillo

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Supporting Information

ABSTRACT: The relationship between the agronomic parameters of grapevine and the phenolic composition of skin of *Vitis vinifera* L. cv. Tempranillo grapes was assessed. The physical and mechanical properties of berries and their skins were also determined and correlated to the chemical composition. Results showed a significant negative correlation between grapevine vigor-related parameters (such as leaf area and bunch weight) and anthocyanin composition, whereas the percentage (w/w) of seeds was negatively correlated with the amount of flavanols of grape skins. Texture properties of grape skins also showed an important relationship with chemical composition. Berry hardness showed a negative correlation with the coumaroyl-anthocyanin derivatives, but it was positively correlated to skin flavanic composition. Moreover, significant regressions with high coefficients of determination were found between phenolic composition and grapevine vigor-related and texture variables, thus pointing out that these parameters might be useful for estimating the phenolic composition of grape skins.

KEYWORDS: *phenolics, anthocyanins, flavanols, Tempranillo red grapes, HPLC-DAD-MS^n, grapevine vigor, mechanical properties*

INTRODUCTION

Important wine organoleptic properties such as color, bitterness, and astringency are strongly influenced by the phenolic composition of the grapes, which, in turn, also provides important information about the aging potential of wines.[1] Anthocyanins, which are extracted from grape skins, are the compounds mainly responsible for wine color. In grapes, not only are the monoglucosides of anthocyanidins present, but also the acetyl, caffeoyl, and *p*-coumaroyl derivatives and even other unusual glycoside derivatives, such as galactosides.[2] In the Tempranillo cultivar, monoglucosides are the main anthocyanins, and acetic acid and *p*-coumaric acid are the most common acids esterifying the glucose moiety.[3] Although monoglucosides of anthocyanidins are the major pigments, acyl derivatives can play an important role in wine color stability because acylation can be related to an increase of the anthocyanidin stability against light, temperature, or pH changes.[4] Moreover, the presence of a cinnamic acid, such as *p*-coumaric or caffeic acid, in the structure can favor intramolecular copigmentation processes and, as a consequence, changes in anthocyanin color in comparison with the original nonacylated pigment.[5]

Flavanols are related to wine astringency and bitterness,[6] although they can also play an important role in long-term color stability.[7] Grape flavanols slightly differ in their structure and in their organoleptic properties according to their origin. Flavanols from grape seed derive from (epi)catechin and show higher levels of galloylation, whereas grape skin contains both catechins and gallocatechins and the corresponding derived proanthocyanidins.[8,9] Furthermore, flavanol galloylation has been associated with more tannic and coarse notes in wine,[10] whereas higher levels of prodelphinidins in wines have as a consequence a reduction of these negative perceptions.[11] Moreover, Kennedy[12] has pointed out that winemakers prefer winemaking procedures leading to an increase of flavanol levels from skins and to less extraction from seeds.

Accumulation of phenolic compounds in red grapes takes place gradually during ripening,[13] and their content at harvest considerably depends on cultivar, agronomical practices, canopy microclimate, and bunch exposure.[14−16] It has been reported in the literature that as vine vigor decreased, total soluble solids in grapes, total phenolics, and anthocyanin contents in wines increased.[17,18] In particular, Cortell and coworkers[19] have reported greater anthocyanin accumulation in low-vigor grapevines and significant increases in skin flavanol contents in berries harvested from zones with a reduction in vine vigor. However, it seems that vine vigor has no significant influence on the flavanol concentration in seeds.[20] Furthermore, although grapevine vigor is mainly related to climatic conditions, the occurrence of important differences in grapevine vigor has been documented even for an established vineyard with identical grape variety, age, and vineyard management practices. These differences have been related to variations in topography and physical and chemical character-

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Table 1. Variables

name of variable	meaning of variable	name of variable	meaning of variable
	anthocyanins (mg/g of skin)		**phenolic acids (mg/g of skin)**
Dp	total delphinidin derivatives	a_caftaric	total caftaric acids
Cy	total cyanidin derivatives	a_coutaric	total coutaric acids
Pt	total petunidin derivatives	a_fertaric	total fertaric acids
Pn	total peonidin derivatives	a_caffeic	total caffeic acids
Mv	total malvidin derivatives	a_coumaric	total coumaric acids and their glucoside derivatives
Monoglc	total anthocyanin monoglucosides	HC	total hydroxycinnamic acids
Acet	total anthocyanin acetylglucosides	HB	total hydroxybenzoic acids
Coumar	total anthocyanin coumaroylglucosides		**agronomic, biophysical, and technological variables**
Caffeo	total anthocyanin caffeoylglucosides	Leaf_area	total leaf area (m^2)
Acyl	total anthocyanin acylglucosides	Fresh_wood	total weight of fresh wood (kg)
Anthoc	total anthocyanins	Dry_wood	total weight of dry wood (kg)
	flavanols (mg/g of skin)	Grape_prod	total weight of bunches (kg)
Cs	catechin and epicatechin	Bunch_weight	average of the weight of bunches (g)
PC_dimer	dimers of procyanidins	Berry_weight	average of the weight of berries (g)
PC_trimer	trimers of procyanidins	Perc_skin	percentage (w/w) of berry skin
PC_tetra	tetramers of procyanidins	Perc_seed	percentage (w/w) of berry seeds
PC_gal	total of galloylated procyanidins	Brix	°Brix of grape must
PC_nongal	total of nongalloylated procyanidins	pH	pH of grape must
PC	total of catechins and procyanidins	Titratable_ac	titratable acidity of must (g/L of tartaric acid)
GCs	gallocatechin and epigallocatechin		**mechanical properties variables**
PD_dimer	dimers of prodelphinidins	Hardness	berry hardness by TPA test (N)
PD_trimers	trimers of prodelphinidins	Gumminess	berry gumminess by TPA test (N)
PD	total of gallocatechins and prodelphinidins	Chewiness	berry chewiness by TPA test (mJ)
PAC	total catechins, gallocatechins and proanthocyanidins	F_{sk}	berry skin break force (N)
		Sp_{sk}	berry skin thickness (μm)

istics of the soil.[20−22] As a result, important differences in the levels of acids, anthocyanins, and phenolics could be found within the same vineyard that can lead to variations in the composition and quality of wines.[23,24]

The numerous physiological and chemical changes that grape berries undergo during grape ripening induce modifications not only in their chemical composition but also in their texture features.[25] These textural modifications have been studied through the evaluation of the grape mechanical properties, which, in turn, have been correlated to grape quality.[26,27] A strong relationship between texture parameters and phenolic ripeness degree and grape variety has been reported.[28−30] In addition, these textural parameters have been demonstrated to be a useful tool to study phenolic extractability from grape skins.[31] However, studies in the literature about the relationship between grapevine-related characteristics, berry mechanical properties, and phenolic composition of grapes are scarce.

Due to the importance of phenolic compounds for wine organoleptic properties, phenolic composition has to be taken into account for the selection of harvest date. However, the harvest date is traditionally and chiefly selected on the basis of the technological maturity of grapes, which is related to the sugar concentration of grapes and therefore determines the alcohol content of wine. Nevertheless, the environmental and climatic conditions may cause technological maturity to be reached before phenolic maturity, and it seems that global climate change is going to increase this delay,[32] making it even more difficult to choose the appropriate harvest date to obtain high-quality wines. For this reason, knowledge about the detailed phenolic composition of grapes can be helpful in establishing strategies for harvest planning.

The purpose of this study was to evaluate the usefulness of parameters related to grapevine vigor and grape texture as indicative tools of the grape skin phenolic composition. Specifically, the main objective of this work was to study the relationship between the phenolic composition of *Vitis vinifera* L. cv. Tempranillo grape skins and the vigor-related grapevine characteristics. In addition, the relationship between texture properties of the berries and their phenolic composition has also been assessed.

MATERIALS AND METHODS

Samples. Thirteen different locations of a vineyard (100 ha) located in Zamora, Spain (coordinates 41°18′26″ N 5°21′45″ W), were selected on the basis of different orographic terrain features, such as orientation, altitude, and slope. For each location, all of the grapes (*V. vinifera* L. cv. Tempranillo) from two different grapevines were collected. All grape samples were collected on the same day at harvest time. Grape samples consisted of 300 berries randomly selected from all collected grapes.

Analysis of Phenolic Composition. Skins were manually separated from berries and extracted following Ferrer-Gallego and co-workers.[33] The detailed phenolic composition of grape skins (mg/g of skin) was analyzed by means of HPLC-DAD-MS. Grape skin extracts were directly analyzed for determining anthocyanin composition, whereas they were fractionated as explained below before analysis of flavanols. In both cases, HPLC analyses were performed in a Hewlett-Packard 1200 series HPLC (Agilent Technologies, Waldbronn, Germany). Mass spectrometry was carried out using an API 3200 Qtrap equipped with an ESI source and a triple-quadrupole linear ion trap mass analyzer that was controlled by Analyst 5.1 software (Applied Biosystems, Darmstadt, Germany). All of the analyses were performed in triplicate.

Anthocyanin composition was determined by using the methodology described by Alcalde-Eon and co-workers.[3] Twenty-three different anthocyanins were identified and quantified and grouped into 11 variables depending on the type of anthocyanidin and the type of anthocyanin derivative (see Table 1). Quantification was performed by HPLC-DAD using external calibration curves of standards of 3-*O*-

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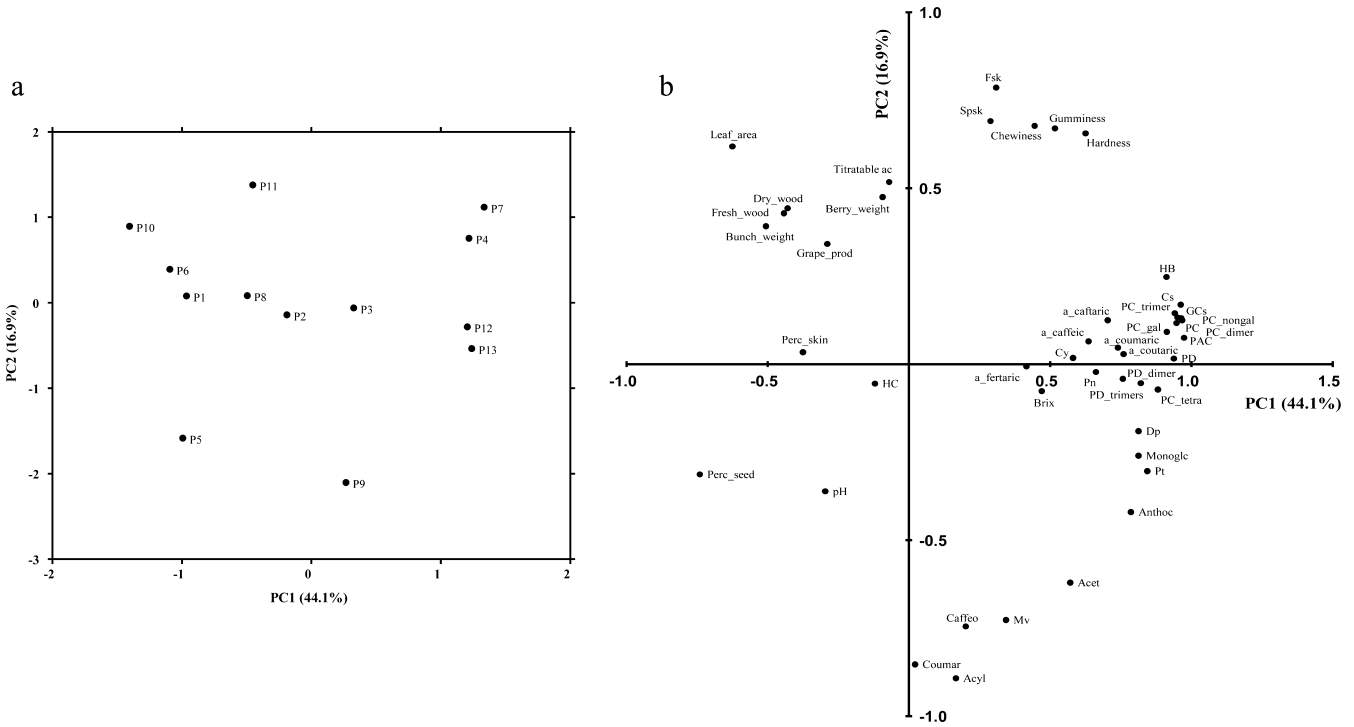

Figure 1. Representation of the samples in the score plot (a) and the variables in the loading plot (b) on the plane defined by the first and second principal components.

glucosides of delphinidin, cyanidin, petunidin, peonidin, and malvidin, purchased from Extrasynthèse (Lyon, France). Each determined anthocyanin was quantified using the calibration curve of the corresponding anthocyanin monoglucoside.

To analyze flavanols and phenolic acids, grape skin extracts were fractionated prior to HPLC-DAD-MS analysis with the objective of eliminating the anthocyanins. Fractionation was carried out according to the procedure described by González-Manzano and co-workers for wine samples.[34] Chromatographic analysis was performed following the methodology reported by Ferrer-Gallego and co-workers.[10] Detection was carried out at 280 nm (proanthocyanidins) and 330 nm (phenolic acids) as the preferred wavelengths. Quantification was performed by HPLC-DAD using external calibration curves of purchased standards except for standards of dimeric and trimeric procyanidins, which were isolated in our laboratory as described by González-Manzano and co-workers.[34] Nineteen different flavanols were determined and grouped into 12 variables depending on the type of flavanol and the polymerization degree (see Table 1). The calibration curves of catechin, dimeric procyanidin, and trimeric procyanidin were employed for quantifying catechin and epicatechin, dimeric procyanidins, and trimers and tetramers of procyanidins, respectively. Galloylated procyanidins were quantified using the epicatechin 3-*O*-gallate calibration curve, whereas gallocatechins and prodelphinidins were quantified using the gallocatechin calibration curve. Two hydroxybenzoic acids and 11 hydroxycinnamic acids and their tartaric esters or glucosidic derivatives were determined and grouped into seven variables (see Table 1). Hydroxybenzoic acids and hydroxycinnamic acids were quantified using the gallic acid and *p*-coumaric acid calibration curves, respectively.

Biophysical and Technological Variables. Eight different biophysical variables were studied (see Table 1), which were also determined at harvest time for each grapevine selected. Data are the average of the values determined for two grapevines of the same location. Leaf area (m^2) was the total leaf area of grapevine. To calculate this value, the number of long, medium-length, and short vine shoots of each grapevine was determined. Considering that long vine shoots have on average 20 knots with each having 4 big-size leaves, whereas medium-long ones have 12 knots with 3 medium-size leaves each and short vine shoots have 8 knots with 2 small-size leaves each, the total number of leaves of each size could be calculated. The average area of each kind of leaf was determined from the area of 10 leaves of each size, which was used to calculate the total leaf area. The grape production (kg) was the total weight of bunches of each grapevine. The average weight of bunches was calculated as the average of the weight of all bunches collected from the same grapevine. The average weight of berries was calculated from the weight of 50 different berries collected from the same grapevine. Moreover, the percentage (w/w) that skin and seeds represented in berry weight was also measured after manual separation of skin and seeds from berries. Grapevines were also pruned after leaf fall, allowing us to calculate the weight of fresh wood. The pruned wood was then dried for 72 h at 60 °C, and the weight of dried wood was determined.

°Brix and pH were directly measured in the grape must by using an optical refractometer and a pH-meter, respectively. Titratable acidity was calculated after acid−base titration of must employing 0.1 M NaOH and expressed as tartaric acid equivalents (g/L).[35]

Instrumental Mechanical Properties. The mechanical properties of the berries were assessed following the method of Letaief and co-workers.[36] A whole-berry texture profile analysis (TPA) double-compression test was carried out at a test speed of 1 mm/s until 25% of sample deformation (2 s waiting time between compressions), with the hardness (N), gumminess (N), and chewiness (mJ) parameters calculated from the force−distance curve.[36] Berry skin break force (F_{sk}, N) was evaluated with a puncture test on the intact berry performed at a test speed of 1 mm/s until 3 mm of sample deformation,[36] whereas the berry skin thickness (Sp_{sk}, μm) was assessed with a 0.2 mm/s compression of a piece of skin using a 2 mm flat cylindrical probe.[36] These parameters were determined by analyzing 30 randomly selected berries collected from the two grapevines of each location.

Statistical Analysis. Principal component analysis (PCA) was used for data analysis as an unsupervised pattern recognition method. The data matrix was constituted by the values determined for all 46 variables described in Table 1 for each selected location. Correlation analyses were carried out, and Pearson's coefficient and two-tailed *p* values were obtained. Backward stepwise multiple linear regression (MLR) was performed to assess the relationship between phenolic composition and the rest of variables. The coefficient of determination (R^2) and the significance (*p* value, bilateral) of the built models were

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Table 2. Pearson's Coefficients of the Most Important Significant Correlation between Phenolic Composition of Grape Skins and Biophysical, Technological, and Texture Variables[a]

	Perc_seed	Leaf_area	Fresh_wood	Dry_wood	Bunch_weight	Berry_weight	F_{sk}	Hardness
Mv	ns	−0.691**	ns	ns	ns	ns	ns	ns
Monoglc	−0.600*	−0.561*	ns	ns	−0.577*	ns	ns	ns
Coumar	ns	−0.607*	−0.698**	−0.706**	ns	ns	−0.635*	ns
Caffeo	ns	ns	ns	ns	ns	−0.666*	ns	ns
Acyl	ns	−0.660*	−0.682*	−0.692**	ns	ns	−0.589*	ns
Anthoc	ns	−0.652*	ns	ns	−0.586*	ns	ns	ns
GCs	−0.825**	ns	ns	ns	ns	ns	ns	0.699**
PC_gal	−0.616*	−0.563*	ns	ns	ns	ns	ns	0.648*
PC_nongal	−0.792**	ns	ns	ns	ns	ns	ns	0.653*
PC	−0.764**	ns	ns	ns	ns	ns	ns	0.661*
PD	−0.782**	ns	ns	ns	ns	ns	ns	0.630*
PAC	−0.791**	ns	ns	ns	ns	ns	ns	0.660*
HB	−0.723**	ns	ns	ns	ns	ns	ns	0.678*

[a]See Table 1 for further information about variable meaning. ns, *, and ** indicate the level of significance (no significant, $p < 0.05$, and $p < 0.01$, respectively, $n = 26$).

studied. The software package IBM SPSS Statistics v. 21.0 (IBM, Armonk, NY, USA) was used for data processing.

RESULTS AND DISCUSSION

Study of Correlations. PCA was conducted as unsupervised pattern recognition technique to observe relationships between biophysical, technological, and texture variables and those related to phenolic composition. Figure 1 shows the projection of the samples on the plane defined by the first and second principal components and also the corresponding loadings plot. The first principal component (PC1) describes 44.15% of the variability, and the second principal component (PC2) describes 16.93% of the variability. As can be seen in Figure 1a, the distribution of samples into the score plot did not show any important grouping, thus pointing out the important differences among the selected grapevines (see also Table 1 in the Supporting Information), which will allow us to study possible correlations between the variables employed. Figure 1b shows the variables on the loadings plot. It can be observed that there is a strong opposition along PC1 between flavanol composition of grape skins and some of the biophysical variables studied, such as leaf area (Leaf_area), the average weight of bunch (Bunch_weight), the weight of fresh (Fresh_wood) and dry wood (Dry_wood), and the percentage (w/w) of seeds in total grape weight (Perc_seed). The latter variable also showed a clear negative relationship with the total anthocyanin content (Anthoc). Hence, it seems that it might be a negative relationship between the biophysical features of grapevine determined in this work and the phenolic composition of grapes. In the same way, the acyl derivatives of anthocyanins [mainly the coumaroyl derivatives (Coumar)] showed high negative values in PC2, in contrast to texture variables and leaf area, which showed high positive values in this PC. Thus, there also may be a negative relationship not only between the composition on anthocyanin acyl derivatives of grapes and their texture properties but also between the levels of these compounds and the biophysical features of grapevine. Moreover, from the low loading values obtained for °Brix in PC1 and in PC2 (<0.45 and > −0.08, respectively), it seems that this variable barely contributes to explain sample variability. This could be related to similarities on the sugar content (°Brix) of analyzed grapes (see Table 1 in the Supporting Information), which would indicate that all samples were collected at a similar status of technological maturity. However, phenolic composition is crucial for sample differentiation, which may point out important differences in the phenolic maturity of collected samples. These results indicate that grapes collected from the same vineyard at a similar status of technological maturity can show important differences in phenolic ripeness. These differences, as will be explained below, can be related to differences in grapevine vigor.

To assess the significance of these relationships, the correlation between all variables employed in the study was investigated by means of Pearson's coefficient and its significance. Table 2 shows the most important significant correlations between the phenolic composition of grape skins and the rest of the variables considered in this study. The phenolic composition did not show any significant correlations with the percentage (w/w) of skins (data not shown). However, they corroborate the negative relationship between the percentage (w/w) of seeds in relation to the whole grape (Perc_seed) and the flavanic composition of grape skins indicated in the PCA plotting (Figure 1b). This is in accordance with studies in the literature which have reported that skin weight was not a determining factor for anthocyanin potential of the berries but that seed weight seemed to significantly affect the grape composition.[37] All variables related to flavanic composition showed high negative coefficients of Pearson with Perc_seed variable. Among them, the total content of flavanols (PAC), as well as the total content of procyanidins (PC) and prodelphinidins (PD), showed Pearson's coefficients < −0.76. Moreover, these correlations are highly significant ($p < 0.01$). Thus, it seems that the heavier the seed, the lower amounts of flavanols in the skins. It might be possible that synthesis of flavanols in seeds and in skin could be competitive and that the highest weight of the seed reflects higher synthesis rate of flavanols in this part of the berry, at the expense of the synthesis in the grape skin. This negative correlation is also observed between total hydroxybenzoic acids content in grape skin and the percentage (w/w) of seeds. Because one of the two hydroxybenzoic acids (the major one) found in the skin is gallic acid, and this acid is also found in grape seeds, this negative correlation might be also due to the same reasons as those proposed for flavanols.

Total leaf area of grapevine also correlates negatively with phenolic composition of grape skin. Anthocyanin compounds

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presented the highest negative Pearson's coefficients. Malvidin derivatives (Mv) and the acyl-derived anthocyanin (Acyl) levels were the most strongly correlated to leaf area. The acyl-derived, and, in particular, the coumaroyl-anthocyanin derivatives (Coumar), also showed a strong negative relationship with the weight of wood pruned from the grapevine (Fresh_wood and Dry_wood). These two variables, together with leaf area, could be related to vine vigor. Our results are consistent with those recently reported by Song and co-workers[17] which have found that as vine vigor decreased, total soluble solids in grapes and total phenolics and anthocyanins in wines increased, thus pointing out a negative relationship between vine vigor and grape phenolic composition. Moreover, vine vigor could be related to the grapevine water availability that in turn seems to affect the composition of grapes because an excess in water conditions has demonstrated to be more negative for anthocyanin contents than strong deficit conditions.[37]

A significant negative relationship could also be observed (Table 2) between the average weight of bunches (Bunch_weight) and the monoglucoside (Monoglc) and total anthocyanin (Antoc_total) contents. Moreover, the level of anthocyanin caffeoyl derivatives is also strongly correlated ($r = -0.666$, $p < 0.05$) to the average weight of berries (Berry_weight). Therefore, it seems that the heavier the bunches and berries were, the lower were the levels of anthocyanins (both total and monoglucoside and caffeoyl derivatives) the skins and, consequently, the berries showed. These results are in accordance with those reported in the literature showing that the total anthocyanin content (mg/berry) and anthocyanin concentration (mg/kg of berries and in mg/g of skin) were dependent on berry mass variation.[38] Likewise, it seems that the berries in which seeds accounted for a higher weight percentage (Perc_seed) show lower levels of monoglucosides, because a significant negative correlation ($r = -0.600$, $p < 0.05$) between these two variables was observed. It has been reported that berry weight is more related to seed weight than to skin and flesh weight,[37,38] so this might explain why both Berry_weight and Perc_seed variables showed a relationship with anthocyanin composition, whereas no relationship was found with Perc_skin variable. These correlations between physical features of berries and their phenolic composition might be explained because grape development occurs in two main stages. The first stage, comprising the flowering and green berry stages, and maybe even prior to that, during differentiation of the primordia,[39] seems critical in determining berry weight.[38] However, anthocyanin and sugar accumulation takes place in a second stage, from veraison to harvest. Thus, if the first stages were the most important, bunches, berries, and seeds could be heavier but grapes may show lower levels of anthocyanins.

Finally, a strong negative correlation was also observed between the texture features of grape and its phenolic composition. In particular, the berry skin break force (F_{sk}) and the levels of anthocyanidin−coumaroylglucosides ($r = -0.635$, $p < 0.05$) and of total acyl-derived anthocyanin ($r = -0.589$, $p < 0.05$) are negatively correlated (Table 2). These results are in accordance with those reported by Giacosa and co-workers,[40] who have observed in Shiraz grapes significantly lower values of F_{sk} in berries showing higher levels of coumaroyl-anthocyanin derivatives in its composition. These results are also consistent with other studies available in the literature pointing out the potential of the mechanical properties of berry skin (such as F_{sk} and Sp_{sk}) to predict the anthocyanin extractability.[29,31] Moreover, it has also been reported that cell-wall composition affects the anthocyanin extraction; in particular, the presence of higher amounts of glucose, rhamnose, 2-*O*-methylxylose, and lignin in the cell-wall composition would prevent anthocyanin extraction from grape skin.[41] Considering this, there might be a relationship between the cell-wall composition and the levels of coumaroyl-anthocyanin derivatives that may be explained by a possible interaction between the acyl-derived anthocyanins and some components of the grape cell wall, which in turn may determine the texture features of grapes. Further studies about the cell-wall and phenolic composition and texture features of berry skin must be carried out to assess this possibility.

Moreover, a significant positive correlation has been observed between berry hardness and its flavanic composition. It is worth noting the strong correlation between this texture parameter and the level of gallocatechin and epigallocatechin ($r = 0.699$, $p < 0.01$, Table 2). Thus, it seems that berry hardness might be indicative of the levels of flavanols in berry skin. Río Segade and co-workers[30] have reported that break force and thickness of berry skin can be considered mechanical properties adequate for the estimation of the degradability of the skin cell wall. Degradation is related to changes in the structure of the cell wall by depolymerization and formation of new cross-linking bridges[42] and to changes in its composition by loss of galactose and other pectic sugars such as arabinose and rhamnose.[30,43,44] Considering that these texture parameters could be related to cell-wall composition, the correlation found between flavanic composition and berry hardness might be explained, as in the case of acyl-derived anthocyanin, by a specific interaction of flavanols with some cell-wall components. In fact, Ruiz-García and co-workers[45] have pointed out that pectic polysaccharides have an important binding affinity for flavanols, whereas cellulose, due to a low porosity, showed less affinity for these compounds. Thus, both higher levels of flavanols and higher values of hardness of berries might be related to higher levels of cellulose in the cell wall. However, further specific studies about the relationship between cell-wall composition and texture features of berries must be carried out to assess this possibility.

Regression Studies. Considering the aforementioned correlations, different multiple linear regressions (MLR) were carried out to assess the influence of biophysical, technological, and texture variables employed in this work on the phenolic composition of grape skin. A backward-stepwise strategy was employed for MLR, in which all of the considered variables were used at the start of the process and then the least significant one is removed at each step. The model is refitted after each step including only the most significant variables. First, due to the correlation found between the amount of coumaroyl-anthocyanin derivatives and the texture parameters that pointed out a possible relationship between these compounds and cell-wall composition, the variable Coumar was selected as dependent variable, whereas the biophysical, technological, and texture variables described in Table 1 were used as independent variables. Among all of the variables considered, only the dry weight of pruned wood (Dry_wood), the berry skin break force (F_{sk}), and the berry skin thickness (Sp_{sk}) were considered statistically significant ($p < 0.05$) in the fitted final model. The values of the coefficient of determination (R^2), the nonstandardized coefficients (B), and the standardized coefficients (β) were obtained. The coefficient of determination ($R^2 = 0.856$) indicates that the proposed model

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explains 85.6% of the variability of the levels of coumaroyl-anthocyanin derivatives, which supposed a good fit to the data. Table 3 shows the values of the regression constant and of the

Table 3. Results of the MLR Carried Out Using the Level of Coumaroyl-Glucoside Anthocyanins (Up) and of Total Flavanols (Down) as Dependent Variables

Dependent Variable: Coumaroyl-Glucoside Anthocyanins (Coumar, mg/g of Skin), R^2 = 0.856			
variable	nonstandardized coefficients (B)	standardized coefficients (β)	p value
Constant	1.934		<0.001
Dry_wood (kg)	−0.333	−0.741	<0.001
F_{sk} (N)	−0.715	−0.300	0.008
Sp_{sk} (mm)	−0.002	−0.352	0.006
Dependent Variable: Total Flavanols (PAC, mg/g of Skin), R^2 = 0.829			
variable	nonstandardized coefficients (B)	standardized coefficients (β)	p value
Constant	2.664		0.020
Leaf_area (m^2)	−8.331	−0.406	0.019
Perc_seed	−0.335	−0.507	0.001
Hardness (N)	0.181	0.310	0.009

β parameter for each variable, which could be considered the best estimation about its contribution to the model. As can be observed in the study of correlations, these three variables (Dry_wood, F_{sk} and Sp_{sk}) showed a negative relationship with the levels of coumaroyl-anthocyanin derivatives. The most important variable in the study was Dry_wood (β = −0.741), thus indicating the importance of grapevine vigor in the levels of these anthocyanin-derived compounds in grapes.

Considering the important role of flavanols in some organoleptic properties of wines such as astringency or color, MLR was also performed using the levels of total flavanols (PAC) as dependent variable and the biophysical, technological, and texture variables described in Table 1 as independent variables. Table 3 shows the result of fitting. The proposed model explained 82.9% of the variability of total flavanol levels (R^2 = 0.829), which indicates the goodness of data fitting. As can be observed in Table 3, the percentage (w/w) of seeds and leaf area showed a negative relationship, whereas berry hardness showed a positive relationship with flavanol content. The most important variable in this model is the percentage (w/w) of seeds, thus pointing out the importance of seed size on the flavanic composition of grape skins.

The proposed models indicated that there is a strong relationship between the biophysical parameters of grapevine (mostly vine vigor represented by leaf area, dry weight of pruned wood, and seed weight), the texture features (evaluated as instrumental mechanical properties) of berries, and the phenolic composition of grape skins. Although this study has been carried out in only one vintage, we have chosen a vineyard large enough to have important differences in orographic terrain features. This could be observed in the PCA and also in the high variability of variables that have been used in this work (see Table 1 in the Supporting Information). Thus, the results here presented set an important precedent because they establish the importance of agronomic parameters and texture properties for estimating the phenolic composition of grape skins. However, further studies involving different vineyards, grape cultivars, and vintages must be done to corroborate the quantitative relationship between these variables.

In conclusion, the results obtained pointed out an important relationship between the phenolic composition of grape skin, biophysical features of grapevines, and berry texture properties. Anthocyanin composition showed significant negative correlation with grapevine vigor-related parameters (such as leaf area and bunch weight), whereas the amount of flavanols of grape skins was negatively correlated with the percentage (w/w) of seeds. Moreover, the phenolic composition is also correlated to some mechanical properties of grapes. Berry skin break force showed a negative correlation with the coumaroyl-anthocyanin derivatives, whereas berry hardness was positively correlated to flavanic composition. Thus, a relationship between both acyl-derived anthocyanins and flavanols and grape cell-wall composition could be proposed. A significant regression was found between coumaroyl-anthocyanin derivatives and some biophysical (weight of pruned wood) and texture (berry skin break force and berry skin thickness) variables. Likewise, a significant regression was also found between flavanol levels and the percentage (w/w) of seeds, leaf area, and berry hardness. These results pointed out that grapevine vigor-related and texture parameters might be useful for estimating the phenolic composition of grape skins.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Minimum, maximum, and average values and coefficients of variation of all the variables employed in this study. The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jafc.5b00275.

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Notes

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